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(54) Title: CDR GRAFTED HUMANISED CHIMERIC T-CELL ANTIBODIES

(57) Abstract

A humanised antibody is provided in which the amino acid sequence of the CDRs is derived from the sequence of the CDRs of a monoclonal antibody having the specificity of binding to resting and activated T-cells, inhibiting T-cell proliferation and lysing T-cells from mice transgenic for human CD2 and in which sufficient of the amino acid sequence of each CDR has been retained to provide the same specificity for the humanised antibody.

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CDR GRAFTED HUMANISED CHIMERIC T-CELL ANTIBODIES

The present invention relates to a humanized antibody which binds to resting and activated T cells, inhibits T cell proliferation and lyses T cells from mice transgenic for human CD2, to the preparation of such an antibody and to a pharmaceutical composition which contains the antibody.

Antibodies typically comprise two heavy chains linked together by disulphide bonds and two light chains. Each light chain is linked to a respective heavy chain by disulphide bonds. Each heavy chain has at one end a variable domain followed by a number of constant domains. Each light chain has a variable domain at one end and a constant domain at its other end. The light chain variable domain is aligned with the variable domain of the heavy chain. The light chain constant domain is aligned with the first constant domain of the heavy chain. The constant domains in the light and heavy chains are not involved directly in binding the antibody to antigen.

The variable domains of each pair of light and heavy chains form the antigen binding site. The domains on the light and heavy chains have the same general structure and each domain comprises a framework of four regions, whose sequences are relatively conserved, connected by three complementarity determining regions (CDRs). The four framework regions largely adopt a beta-sheet conformation and the CDRs form loops connecting, and in some cases forming part of, the beta-sheet structure. The CDRs are held in close proximity by the framework regions and, with the CDRs from the other domain, contribute to the formation of the antigen binding site. CDRs and framework regions of antibodies may be determined by reference to Kabat et al., ("Sequences of proteins of immunological interest" US Dept.

of Health and Human Services, US Government Printing Office, 1987).

The preparation of an altered antibody in which the CDRs are derived from a different species than the framework of the antibody's variable domains is disclosed in EP-A-0239400. The CDRs may be derived from a rat or mouse monoclonal antibody. The framework of the variable domains, and the constant domains, of the altered antibody may be derived from a human antibody. Such a humanised antibody elicits a negligible immune response when administered to a human compared to the immune response mounted by a human against a rat or mouse antibody. Humanised CAMPATH-1 antibody (Campath is a Trademark of The Wellcome Foundation Ltd.) is disclosed in EP-A-0328404.

Human T cells play an important role in regulation of the immune response. Anti-T cell antibodies may therefore be immunosuppressive when administered *in vivo*. Such antibodies may be useful as a result in the treatment of for example, graft versus host disease, transplant rejection and autoimmune diseases such as rheumatoid arthritis.

Non-human monoclonal antibodies have been raised which are anti-T cell antibodies. However, non-human monoclonal antibodies do not fix human complement particularly well and are immunogenic when injected into a human patient. Chimaeric antibodies have been proposed in WO 89/09622 which are composed of a human constant region and a mouse variable region. However, a significant immunogenicity problem remains.

According to one aspect the present invention provides a humanised antibody in which the amino acid sequence of the CDRs is derived from the sequence of the CDRs of a monoclonal antibody having the specificity of

binding to resting and activated T-cells, inhibiting T-cell proliferation and lysing T-cells from mice transgenic for human CD2 and in which sufficient of the amino acid sequence of each CDR has been retained to provide the same specificity for the humanised antibody.

According to another aspect of the present invention, there is provided a humanised antibody in which sufficient of the amino acid sequence of each CDR shown below is provided such that the antibody is capable of binding to a human T-cell antigen:

light chain:	CDR1	(SEQ ID NOS: 3 and 4)
	CDR2	(SEQ ID NOS: 5 and 6)
	CDR3	(SEQ ID NOS: 7 and 8)
heavy chain:	CDR1	(SEQ ID NOS: 11 and 12)
	CDR2	(SEQ ID NOS: 13 and 14)
	CDR3	(SEQ ID NOS: 15 and 16)

The antibody preferably has the structure of a natural antibody or a fragment thereof. The antibody may therefore comprise a complete antibody, a (Fab')2 fragment, a Fab fragment, a light chain dimer or a heavy chain dimer. The antibody may be an IgG such as IgG1, IgG2, IgG3 or IgG4; or IgM, IgA, IgE or IgD. The constant domain of the antibody heavy chain may be selected accordingly. The light chain constant domain may be a kappa or lambda constant domain.

The antibody may be a chimaeric antibody of the type described in WO 86/01533. A chimaeric antibody according to WO 86/01533 comprises an antigen binding region and a non-immunoglobulin region. The antigen binding region is an antibody light chain variable domain and/or heavy chain variable domain. Typically the chimaeric antibody comprises both light and heavy chain variable domains. The

non-immunoglobulin region is fused to the C-terminus of the antigen binding region. The non-immunoglobulin region is typically a non-immunoglobulin protein and may be an enzyme region, a region derived from a protein having known binding specificity, from a protein toxin or indeed from any protein expressed by a gene. The non-immunoglobulin region may be a carbohydrate region. The two regions of the chimaeric antibody may be connected via a cleavable linker sequence.

The light chain CDRs 1 to 3 and heavy chain CDRs 1 to 3 of Seq ID Nos: 3 to 8 and Seq ID Nos: 11 to 16 respectively are the CDRs of the anti-human T cell antibody YTH 655(5)6. YTH 655(5)6 is a rat IgG2b monoclonal antibody which binds to resting and activated T cells, inhibits T cell proliferation and lyses T cells from mice transgenic for human CD2. The specificity of a humanized YTH 655 antibody can be determined by its ability to bind to resting and activated T cells, inhibit T cell proliferation and lyse T cells from mice transgenic for human CD2.

Suitably, the CDRs of a humanised antibody are the light chain CDRs 1 to 3 and the heavy chain CDRs 1 to 3 above. The amino acid sequences of these CDRs may be changed, however. The amino acid sequence of each CDR may be changed by up to 40% by amino acid substitutions, insertions and/or deletions, for example by up to 30%, up to 20% or up to 10%.

Each CDR may therefore include one or two amino acid substitutions, insertions and/or deletions. There may be up to three amino acid substitutions, insertions and/or deletions in light chain CDR3 or heavy chain CDR3. Up to four amino acid substitutions, insertions and/or deletions may be present in light chain CDR1. Up to six amino acid substitutions, insertions and/or deletions may be present

in heavy chain CDR2. Preferably the amino acid sequence of each CDR is substantially homologous to that of each CDR of the anti-T cell antibody YTH 655(5)6.

The framework and the constant domains of the antibody are human framework and human constant domains. Preferably the framework of the variable region of the antibody heavy chain is substantially homologous to the corresponding framework of the human protein KOL (Schmidt et al., Hoppe-Seyler's Z. Physiol. Chem., 364 713-747, 1983). Homology in respect of the framework is generally 80% or more with respect to KOL, for example 90% or more or 95% or more. A number of amino acid substitutions, insertions and/or deletions may be present. For example, the seventh residue of framework 4 is suitably Thr or Leu, preferably Leu. This residue is KOL residue 109 by Kabat et al., 1987. Other candidate framework changes that may be made to restore binding include amino acid residues 27, 30, 48, 66, 67, 71, 91, 93 and 94. The amino acid numbering is according to Kabat et al.

The framework of the variable region of the antibody light chain is typically substantially homologous to the variable domain framework of the protein HSIGKVII (EMBL data base: Klobeck, H.G., EMBL data library submitted 7th April, 1986). There is a frameshift in this sequence at position 452. To rectify the reading frame, a deletion of base 452(T) was made. Homology in respect of the framework is generally 80% or more with respect to HSIGKVII, for example 90% or more or 95% or more. A number of amino acid substitutions, insertions and/or deletions may be present, for example at amino acid residue 71 according to the numbering of Kabat et al.

A humanised antibody is prepared according to the invention by a process which comprises maintaining a host transformed with a first expression vector which encodes

the light chain of the humanised antibody and with a second expression vector which encodes the heavy chain of the humanised antibody under such conditions that each chain is expressed and isolating the humanised antibody formed by assembly of the thus-expressed chains.

The first and second expression vectors may be the same vector. The invention further provides:

- a DNA sequence encoding the light chain or the heavy chain of the humanised antibody;
- an expression vector which incorporates said DNA sequence(s); and
- a host transformed with a said expression vector.

Each chain of the antibody may be prepared by CDR replacement. The CDRs of a variable region of a light or heavy chain of a human antibody are replaced by sufficient of the amino acid sequence of each CDR of the YTH 655 antibody that the resulting antibody is capable of binding to resting and activated T cells. The CDR-encoding regions of DNA encoding a hypervariable region of a human antibody chain are replaced by DNA encoding the desired CDRs. If appropriate, this altered DNA is linked to DNA encoding a constant domain for the antibody chain. The DNA is cloned into an expression vector. The expression vector is introduced into a compatible host cell which is cultured under such conditions that the antibody chain is expressed. Complementary antibody chains which are co-expressed in this way may then assemble to form the humanised antibody.

There are four general steps to humanise a monoclonal antibody. These are:

(1) determining the nucleotide and predicted amino acid sequence of the starting antibody light and heavy variable domains;

(2) designing the humanised antibody, i.e. deciding which antibody framework region to use during the humanising process;

(3) the actual humanising methodologies/techniques; and

(4) the transfection and expression of the humanised antibody.

Step 1:

Determining the nucleotide and predicted amino acid sequence of the antibody light and heavy chain variable domains

To humanise an antibody only the amino acid sequence of antibody's heavy and light chain variable domains needs to be known. The sequence of the constant domains is irrelevant because these do not contribute to the reshaping strategy. The simplest method of determining an antibody's variable domain amino acid sequence is from cloned cDNA encoding the heavy and light chain variable domain.

There are two general methods for cloning a given antibody's heavy and light chain variable domain cDNAs: (1) via a conventional cDNA library, or (2) via the polymerase chain reaction (PCR). Both of these methods are widely known. Given the nucleotide sequence of the cDNAs, it is a simple matter to translate this information into the predicted amino acid sequence of the antibody variable domains. In the present instance, the nucleotide sequence and predicted amino acid sequence of the rodent YTH 655 antibody chains are shown in SEQ ID NOS: 1 and 2 (light) and SEQ ID NOS: 9 and 10 (heavy).

Step 2:

Designing the humanised antibody

There are several factors to consider in deciding which human antibody sequence to use during the humanisation. The humanisation of light and heavy chains are considered independently of one another, but the reasoning is basically similar for each.

This selection process is based on the following rationale: A given antibody's antigen specificity and affinity is primarily determined by the amino acid sequence of the variable region CDRs. Variable domain framework residues have little or no direct contribution. The primary function of the framework regions is to hold the CDRs in their proper spatial orientation to recognize antigen. Thus the substitution of rodent CDRs into a human variable domain framework is most likely to result in retention of their correct spatial orientation if the human variable domain framework is highly homologous to the rodent variable domain from which they originated. A human variable domain shculd preferably be chosen therefore that is highly homologous to the rodent variable domain(s).

A suitable human antibody variable domain sequence can be selected as follows:

1. Using a computer program, search all available protein (and DNA) databases for those human antibody variable domain sequences that are most homologous to the rodent antibody variable domains. The output of a suitable program is a list of sequences most homologous to the rodent antibody, the percent homology to each sequence, and an alignment of each sequence to the rodent sequence. This is done independently for both the heavy and light chain variable domain sequences. The above analyses are more easily accomplished if only human immunoglobulin

sequences are included.

2. List the human antibody variable domain sequences and compare for homology. Primarily the comparison is performed on length of CDRs, except CDR3 of the heavy chain which is quite variable. Human heavy chains and Kappa and Lambda light chains are divided into subgroups; Heavy chain 3 subgroups, Kappa chain 4 subgroups, Lambda chain 6 subgroups. The CDR sizes within each subgroup are similar but vary between subgroups. It is usually possible to match a rodent antibody CDR to one of the human subgroups as a first approximation of homology. Antibodies bearing CDRs of similar length are then compared for amino acid sequence homology, especially within the CDRs, but also in the surrounding framework regions. The human variable domain which is most homologous is chosen as the framework for humanisation.

Step 3:

The actual humanising methodologies/techniques

An antibody may be humanised by grafting the desired CDRs onto a human framework according to EP-A-0239400. A DNA sequence encoding the desired reshaped antibody can therefore be made beginning with the human DNA whose CDRs it is wished to reshape. The rodent variable domain amino acid sequence containing the desired CDRs is compared to that of the chosen human antibody variable domain sequence. The residues in the human variable domain are marked that need to be changed to the corresponding residue in the rodent to make the human variable region incorporate the rodent CDRs. There may also be residues that need substituting in, adding ~~to or deleting~~ from the human sequence.

Oligonucleotides are synthesized that can be used to mutagenize the human variable domain framework to contain the desired residues. Those oligonucleotides can be of any convenient size. One is normally only limited in length by the capabilities of the particular synthesizer one has available. The method of oligonucleotide-directed *in vitro* mutagenesis is well known.

Alternatively, humanisation may be achieved using the recombinant polymerase chain reaction (PCR) methodology of WO 92/07075. Using this methodology, a CDR may be spliced between the framework regions of a human antibody.

In general, the technique of UK Application No. 9022011.2 can be performed using a template comprising two human framework regions, AB and CD, and between them, the CDR which is to be replaced by a donor CDR. Primers A and B are used to amplify the framework region AB, and primers C and D used to amplify the framework region CD. However, the primers B and C each also contain, at their 5′ ends, an additional sequence corresponding to all or at least part of the donor CDR sequence. Primers B and C overlap by a length sufficient to permit annealing of their 5′ ends to each other under conditions which allow a PCR to be performed. Thus, the amplified regions AB and CD may undergo gene splicing by overlap extension to produce the humanised product in a single reaction.

Step 4:

The transfection and expression of the reshaped antibody

Following the mutagenesis reactions to reshape the antibody, the mutagenised DNAs can be linked to an appropriate DNA encoding a light or heavy chain constant region, cloned into an expression vector, and transfected into host cells, preferably mammalian cells. These steps

can be carried out in routine fashion. A reshaped antibody may therefore be prepared by a process comprising:

(a) preparing a first replicable expression vector including a suitable promoter operably linked to a DNA sequence which encodes at least a variable domain of an Ig heavy or light chain, the variable domain comprising framework regions from a human antibody and the CDRs required for the humanised antibody of the invention;

(b) preparing a second replicable expression vector including a suitable promoter operably linked to a DNA sequence which encodes at least the variable domain of a complementary Ig light or heavy chain respectively;

(c) transforming a cell line with the first or both prepared vectors; and

(d) culturing said transformed cell line to produce said altered antibody.

Preferably the DNA sequence in step (a) encodes both the variable domain and the or each constant domain of the human antibody chain. The humanised antibody can be recovered and purified. The cell line which is transformed to produce the altered antibody may be a Chinese Hamster Ovary (CHO) cell line or an immortalised mammalian cell line, which is advantageously of lymphoid origin, such as a myeloma, hybridoma, trioma or quadroma cell line. The cell line may also comprise a normal lymphoid cell, such as a B-cell, which has been immortalised by transformation with a virus, such as the Epstein-Barr virus. Most preferably, the immortalised cell line is a myeloma cell line or a derivative thereof.

Although the cell line used to produce the humanised antibody is preferably a mammalian cell line, any other suitable cell line, such as a bacterial cell line or a yeast cell line, may alternatively be used. For single antibody chains, it is envisaged that *E. coli* - derived bacterial strains could be used. The antibody obtained is checked for functionality. If functionality is lost, it is necessary to return to step (2) and alter the framework of the antibody.

Once expressed, the whole antibodies, their dimers, individual light and heavy chains, or other immunoglobulin forms of the present invention can be purified according to standard procedures of the art, including ammonium sulfate precipitation, affinity columns, column chromatography, gel electrophoresis and the like (see, generally, Scopes, R., Protein Purification, Springer-Verlag, N.Y. (1982)). Substantially pure immunoglobulins of at least about 90 to 95% homogeneity are preferred, and 98 to 99% or more homogeneity most preferred, for pharmaceutical uses. Once purified, partially or to homogeneity as desired, a humanised antibody may then be used therapeutically or in developing and performing assay procedures, immunofluorescent stainings, and the like (see, generally, Immunological Methods, Vols. I and II, Lefkovits and Pernis, eds., Academic Press, New York, N.Y. (1979 and 1981)).

The Human T cell antigen specific antibodies typically find use in treating a T-cell mediated disease state. Generally, where the cell linked to a disease has been identified as bearing the T cell antigen, then the humanised antibodies capable of binding the T cell antigen are suitable. For example, typical disease states suitable for treatment include graft versus host disease and transplant rejection in patients undergoing an organ transplant, such as heart, lungs, kidneys, liver, etc.

Other diseases include autoimmune diseases, such as Type I diabetes, multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosus, and myasthenia gravis.

The human-like antibodies of the present invention may also be used in combination with other antibodies, particularly human monoclonal antibodies reactive with other markers on cells responsible for the disease. For example, suitable T-cell markers can include those grouped into the so-called "Clusters of Differentiation," as named by the First International Leukocyte Differentiation Workshop, Leukocyte Typing, Bernard, et al., Eds., Springer-Verlag, N.Y. (1984).

The antibodies can also be used as separately administered compositions given in conjunction with chemotherapeutic or immunosuppressive agents. Typically, the agents will include cyclosporin A or a purine analog (e.g., methotrexate, 6-mercaptopurine, or the like), but numerous additional agents (e.g., cyclophosphamide, prednisone, etc.) well-known to those skilled in the art may also be utilized.

An antibody of the present invention may form part of an immunotoxin. Immunotoxins are characterized by two components and are particularly useful for killing selected cells *in vitro* or *in vivo*. One component is a cytotoxic agent which is usually fatal to a cell when attached or absorbed. The second component, known as the "delivery vehicle", provides a means for delivering the toxic agent to a particular cell type, such as cells comprising a carcinoma. The two components are commonly chemically bonded together by any of a variety of well-known chemical procedures. For example, when the cytotoxic agent is a protein and the second component is an intact immunoglobulin, the linkage may be by way of heterobifunctional cross-linkers, e.g., SPDP, carbodiimide,

glutaraldehyde, or the like. Production of various immunotoxins is well-known with the art, and can be found, for example in "Monoclonal Antibody-Toxin Conjugates: Aiming the Magic Bullet", Thorpe et al., Monoclonal Antibodies in Clinical Medicine, Academic Press, pp. 168-190 (1982).

A variety of cytotoxic agents are suitable for use in immunotoxins. Cytotoxic agents can include radionuclides, such as Iodine-131, Yttrium-90, Rhenium-188, and Bismuth-212; a number of chemotherapeutic drugs, such as vindesine, methotrexate, adriamycin, and cisplatin; and cytotoxic proteins such as ribosomal inhibiting proteins like pokeweed antiviral protein, Pseudomonas exotoxin A, ricin, diphtheria toxin, ricin A chain, etc., or an agent active at the cell surface, such as the phospholipase enzymes (e.g., phospholipase C). See, generally, "Chimeric Toxins," Olsnes and Phil, Pharmac. Ther., 25:335-381 (1982), and "Monoclonal Antibodies for Cancer Detection and Therapy," eds. Baldwin and Byers, pp. 159-179, 224-266, Academic Press (1985).

The delivery component of the immunotoxin is a humanised antibody according to the present invention. Intact immunoglobulins or their binding fragments, such as Fab, are preferably used. Typically, the antibodies in the immunotoxins will be of the human IgA, IgM or IgG isotype, but other mammalian constant regions may be utilized as desired.

The invention further provides a pharmaceutical composition comprising a pharmaceutially acceptable carrier or diluent and, as active ingredient, a humanised antibody according to the invention. The composition may comprise an immunotoxin according to the invention. The humanised antibody, immunotoxin and pharmaceutical compositions thereof of this invention are particularly useful for

parenteral administration, i.e., subcutaneously, intramuscularly or intravenously.

The compositions for parenteral administration will commonly comprise a solution of the antibody or a cocktail thereof dissolved in an acceptable carrier, preferably an aqueous carrier. A variety of aqueous carriers can be used, e.g., water, buffered water, 0.4% saline, 0.3% glycine and the like. These solutions are sterile and generally free of particulate matter. These compositions may be sterilized by conventional, well known sterilization techniques. The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents, toxicity adjustment agents and the like, for example sodium acetate, sodium chloride, potassium chloride, calcium chloride, sodium lactate, etc. The concentration of antibody in these formulations can vary widely, for example from less than about 0.5%, usually at or at least about 1% to as much as 15 or 20% by weight and will be selected primarily based on fluid volumes, viscosities, etc., in accordance with the particular mode of administration selected.

Thus, a typical pharmaceutical composition for intramuscular injection could be made up to contain 1 ml sterile buffered water, and 50 mg of antibody. A typical composition for intravenous infusion could be made up to contain 250 ml of sterile Ringer's solution, and 150 mg of antibody. Actual methods for preparing parenterally administrable compositions will be known or apparent to those skilled in the art and are described in more detail in, for example, Remington's Pharmaceutical Science, 15th ed., Mack Publishing Company, Easton, Pennsylvania (1980).

The antibodies of this invention can be lyophilized for storage and reconstituted in a suitable carrier prior

to use. This technique has been shown to be effective with conventional immune globulins. Any suitable lyophilization and reconstitution techniques can be employed. It will be appreciated by those skilled in the art that lyophilization and reconstitution can lead to varying degrees of antibody activity loss (e.g., with conventional immune globulins, IgM antibodies tend to have greater activity loss than IgG antibodies) and that use levels may have to be adjusted to compensate.

The compositions containing the present human-like antibodies or a cocktail thereof can be administered for prophylactic and/or therapeutic treatments. In therapeutic application, compositions are administered to a patient already suffering from a disease, in an amount sufficient to cure or at least partially arrest or alleviate the disease and its complications. An amount adequate to accomplish this is defined as a "therapeutically effective dose." Amounts effective for this use will depend upon the severity of the infection and the general state of the patient's own immune system, but generally range from about 1 to about 200 mg of antibody per dose, with dosages of from 5 to 25 mg per patient being more commonly used. It must be kept in mind that the materials of the invention may generally be employed in serious disease states, that is life-threatening or potentially life-threatening situations. In such cases, in view of the minimization of extraneous substances and the lower probability of "foreign substance" rejections which are achieved by the present human-like antibodies of this invention, it is possible and may be felt desirable by the treating physician to administer substantial excesses of these antibodies.

In prophylactic applications, compositions containing the present antibodies or a cocktail thereof are administered to a patient not already in a disease state to enhance the patient's resistance. Such an amount is

defined to be a "prophylactically effective dose". In this use, the precise amounts again depend upon the patient's state of health and general level of immunity, but generally range from 0.1 to 25 mg per dose, especially 0.5 to 2.5 mg per patient. A preferred prophylactic use is for the prevention of kidney transplant rejection.

Single or multiple administrations of the compositions can be carried out with dose levels and pattern being selected by the treating physician. In any event, the pharmaceutical formulations should provide a quantity of the antibody(ies) of this invention sufficient to effectively treat the patient.

Human-like antibodies of the present invention can further find a wide variety of utilities *in vitro*. By way of example, the exemplary antibodies can be utilized for T-cell typing, for isolating specific YTH 655 antigen bearing cells or fragments of the receptor, for vaccine preparation, or the like.

For diagnostic purposes, the antibodies may either be labelled or unlabelled. Unlabelled antibodies can be used in combination with other labelled antibodies (second antibodies) that are reactive with the humanised antibody, such as antibodies specific for human immunoglobulin constant regions. Alternatively, the antibodies can be directly labelled. A wide variety of labels may be employed, such as radionuclides, fluors, enzymes, enzyme substrates, enzyme cofactors, enzyme inhibitors, ligands (particularly haptens), etc. Numerous types of immunoassays are available and are well known to those skilled in the art.

Kits can also be supplied for use with the subject antibodies in the protection against or detection of a cellular activity or for the presence of a selected

antigen. Thus, a humanised antibody of the present invention may be provided, usually in a lyophilized form in a container, either alone or in conjunction with additional antibodies specific for the desired cell type. The antibodies, which may be conjugated to a label or toxin, or unconjugated, are included in the kits with buffers, such as Tris, phosphate, carbonate, etc., stabilizers, biocides, inert proteins, e.g., serum albumin, or the like, and a set of instructions for use. Generally, these materials will be present in less than about 5% wt. based on the amount of active antibody, and usually present in total amount of at least about 0.001% wt. based again on the antibody concentration. Frequently, it will be desirable to include an inert extender or excipient to dilute the active ingredients, where the excipient may be present in from about 1 to 99% wt. of the total composition. Where a second antibody capable of binding to the chimeric antibody is employed in an assay, this will usually be present in a separate vial. The second antibody is typically conjugated to a label and formulated in an analogous manner with the antibody formulations described above.

Figure 1.

Binding of Humanized YTH 655 to MF14 cells.

The activity of humanized YTH 655 (HUMCD2) was assayed by FACS using an activated T cell line called MF14. A chimeric YTH 655 (CHIMCD2) containing a human IgG1 constant region and YTH 655 variable regions was used as a control. Cells were first incubated with either chimeric YTH 655 or humanized YTH 655. After washing, the cells were incubated with a commercially available anti-human FITC then analyzed by FACS. The figure shows that the binding of humanized YTH 655 is equivalent to binding of chimeric YTH 655 and that the humanized YTH 655 binding can be titrated. The antigen specificity of the humanized

monoclonal antibody, therefore, has been retained.

The following Example illustrates the invention.

Cloning and Sequencing of YTH 655 antibody heavy chain

A cDNA encoding the VH region of the YTH 655 antibody was isolated by a polymerase chain reaction (PCR)-based method (Orlandi et al., PNAS USA, 86: 3833-3837, 1989) with some modifications. Total RNA was isolated from hybridoma cells by the guanidine thiocyanate method (Chirgwin et al., Biochemistry, 18: 5294, 1979), and poly $(A)^+$ RNA was isolated by passage of total RNA through, and elution from a poly (U) sepharose 4B column (Pharmacia, Milton Keynes, U.K.). For first strand synthesis, 5ug poly $(A)^+$ RNA was combined with 250uM each dNTP, 10mM dithiothreitol, 50mM Tris.HCl (pH8.2 at 42°C), 10mM MgCl2, 100mM KC1, 10pmoles of the V_H region-specific primer VH_1FOR[5'-d(TGA GGA GAC GGT GAC CGT GGT CCC TTG GCC CCA G] and diethyl pyrocarbonate (DEPC) - treated distilled water to 24ul. This was heated to 70°C for 10 minutes, then 42°C for 10 minutes before adding 23 units Super RT (AMV reverse transcriptase; Anglia Biotec, Colchester, UK). The reaction was carried out at 42°C for 1 hour.

Subsequent 50ul PCR amplifications consisted of 5ul of the first strand synthesis reaction (unpurified), 500uM each dNTP, 67mM Tris-HC1 (pH8.8 at 25°C), 17mM $(NH_4)_2SO_4$, 10mM $MgCl_2$, 20ug/ml gelatin, 5 units TAQ DNA polymerase (Koch-Light, Haverhill, U.K.), 25pmoles of primer VH_1FOR and 25pmoles of the mixed primer VH_1BACK[5'-d(AGG T(CG)(CA) A(GA)CTGC AG(GC) AGT C(TA)G G]. Reactions were overlayed with mineral oil and subjected to 30 cycles of 1.5 minutes at 95°C (denaturation), 3 minutes at 50°C (annealing) and 3 minutes at 72°C (extension) with a Techne PHC-1 programmable cyclic reactor. The final cycle contained a 10 minute extension time.

The sample was frozen at -20°C and the mineral oil (a viscous liquid at -20°C) was removed by aspiration. The aqueous phase was thawed and, after electrophoresis through 2% agarose, a 350bp PCR product was gel-purified. The PCR product was double-digested with PstI and BstEII. Initially this was cloned into the Pstl and BstEII restriction sites of the vector M13VH PCR1 (Orlandi et al., 1989). However, on sequencing resulting clones by the dideoxy chain termination method (Sanger et al., PNAS USA 74: 5463-5467, (1977), the YTH 655 VH gene was found to contain an internal PstI restriction site situated in the framework region between CDR2 and CDR3. An alternative cloning procedure was undertaken whereby the PCR product was digested with PstI only and cloned into the PstI site of M13mp18 (Yanisch-Perron et al., Gene 33, 103-119, 1985). The complete VH gene was subsequently reconstructed by isolating the PstI fragment from M13mp18 and cloning it into the PstI site of M13VHPCR1 (containing the VH PstI-BstEII fragment). The correct orientation of the PstI fragment was determined by dideoxy sequence analysis. Finally, to ensure that the YTH 655 VH gene contains only one internal Pstl site (i.e. that no DNA had been lost as a consequence of the step-wise cloning procedure) a 60bp fragment encompassing this site was cloned and sequenced. The 60bp fragment was generated by XmnI-Bg1II double digestion of the VH PCR product and was then cloned into the HincII-BamHI sites, respectively, of M13mp19.

Nucleotide sequence analysis of random VH PCR products from independent PCR amplifications, and independent RNA isolations, revealed a single species of VH region cDNA. The cDNA sequence and the predicted amino-acid sequence are shown below. As no additional VH region-encoding clones were found, it was assumed that this sequence was derived from the YTH 655 antibody gene.

Cloning and Sequencing of YTH 655 antibody light chain

Total RNA was isolated from hybridoma cells by the guanidine thiocyanate method (Chirguwin et al., Biochemistry, 18, 5294, 1979). Dynabeads Oligo $(dT)_{25}$ (Dynal) was used to extract mRNA from total RNA employing the manufacturer's protocol.

cDNA was synthesised from the isolated mRNA and cloned into the plasmid pSPORT-1 using the Superscript Plasmid System for cDNA Synthesis and Plasmid Cloning kit (BRL) following the method recommended by the manufacturer. Eschericia coli, Max Efficiency DH5α Competent Cells (BRL) were transformed with the resulting cDNA/pSPORT-1 ligation. Approximately 5000 colonies were lifted onto Hybond-N nylon filters (Amersham) and lysed, denatured and fixed following the method of Buluwela et al., (Nucleic Acids Res. 17, 452, 1989). The filters were treated with proteinase K (50μg/ml) in 0.2 x SSC, 0.1% SDS at 55°C for 30 minutes and then excess debris removed with a tissue.

M13 phage supernatant with truncated light chain was used to make a probe to screen the filters. The M13 phage supernatant was PCR'd using M13 reverse and universal primers and 2μl of $^{32}_{\alpha}$ P-ATP. The filters were screened using 25μl of the radioactive probe in the hybridization solution according to the method of Church and Gilbert (PNAS, 81, 1991-1995, 1982). Approximately 30 potential positive colonies were detected. Plasmid DNA was prepared from the positive clones by the method of Del Sal et al., (Nucleic Acids Research 16, 9878, 1988). The DNA was restricted with Not I and Sal I then analysed by Southern blot using the ^{32}P M13 phage supernatant probe previously described. Four positive clones were sequenced using T7, T3 and framework 4 primers following the dideoxy chain termination method (Sanger et al., PNAS, USA, 74, 5463-5467, 1977). Three clones were truncated and one was full length YTH 655 antibody light chain. The full length clone was sequenced fully using the dideoxy chain

termination method.

Designing the chimaeric antibody

Using the selection procedure described in Step 2 above, the human variable domain frameworks of the KOL heavy chain (Kabat et al., 1987) and HSIGKVII light chain (EMBL data base; Klobeck, H.G. EMBL data library submitted 7th April, 1986) were chosen for the humanisation process.

Construction of the humanised heavy and light chain genes

The humanised heavy and light chains were constructed following the method of Lewis and Crowe (Gene 101, 297-302, 1991).

(i) Light Chain

Light chain oligonucleotide primers:

A_L: SEQ ID NO: 17:
B_L: SEQ ID NO: 18:
C_L: SEQ ID NO: 19:
D_L: SEQ ID NO: 20:
E_L: SEQ ID NO: 21:
F_L: SEQ ID NO: 22:
G_L: SEQ ID NO: 23:
H_L: SEQ ID NO: 24:

PCR reactions (Saiki et al., Science 239, 487-491, 1988) were performed in a programmable heating block (Hybaid) using 20 rounds of temperature cycling (94°C for 1 minute 30 seconds, 50°C for 2 min, and 72°C for 3 min) followed by a final 10 min step at 72°C. 800ng of each primer, a specified amount of template, and 2.5 units of Taq polymerase (Perkin Elmer Cetus) were used in a final volume of 100μl with the reaction buffer as recommended by the manufacturer.

The initial template for the PCR was previously humanized Hum DXC2 light chain, a human kappa light chain with HSIGKVII frameworks which had subsequently undergone site-directed mutagenesis to replace CDRL1, CDRL2, and CDRL3 with rat antidigoxin monoclonal antibody (DX48) CDRL1, CDRL2 and CDRL3.

Four primary PCR reactions were initially carried out, with 10ng of template per reaction, using the primer pairs A_L with B_L, C_L with D_L, E_L with F_L, and G_L with H_L respectively. The products of these PCR reactions, fragments AB_L, CD_L, EF_L and GH_L respectively, were purified using Prep-A-Gene (Bio-Rad) following the protocol recommended by the manufacturer. Fragments AB_L with CD_L, and EF_L with GH_L were combined using a quarter of each purified product, and subjected to recombinant PCR reactions with primers A_L plus D_L, and E_L plus H_L respectively. The products of these reactions, fragments AD_L and EH_L, were purified as above, and a quarter of each combined in a recombinant PCR reaction using primers A_L and H_L. The final humanised light chain recombinant PCR product, AH_L, was cloned into the HindIII site of pUC-18 (BR_L) following the method of Crowe et al., 1991, utilising the HindIII sites in primers A_L and H_L. Plasmid isolates were sequenced by the dideoxy chain termination method, and clones of the correct sequence chosen.

(ii) Heavy Chain

Heavy chain oligonucleotide primers:

A_H: SEQ ID NO: 25:

B_H: SEQ ID NO: 26:

C_H: SEQ ID NO: 27:

D_H: SEQ ID NO: 28:

E_H: SEQ ID NO: 29:

F_H: SEQ ID NO: 30:

G_H: SEQ ID NO: 31:

H_H: SEQ ID NO: 32:

The initial template for the PCR was humanised anti-CD4 heavy chain (on KOL framework; WO 92/05274; Gorman et al., Proc. Natl. Acad. Sci. USA 88, 1991) subsequently converted from genomic to cDNA context. The rodent CDR's were grafted on to the template following the recombinant PCR method as described above, but using oligonucleotide primers A_H to H_H. Oligonucleotides A_H and H_H were designed with HindIII and EcoRI sites respectively to enable initial cloning of the humanised variable region, and a SpeI site was introduced into the KOL framework 4 (FR4) region of oligonucleotide G_H to facilitate subsequent cloning of the variable region with a suitable constant region of choice. The SpeI site altered the threonine residue at position 109 (numbering according to Kabat et al., 1987) of the humanised anti-CD4 heavy chain template (proline in KOL) to a leucine residue (four out of the six human heavy J-minigenes possess a leucine at this position; Kabat et al., 1987).The humanised heavy chain variable region recombinant PCR product was cloned into HindIII/EcoRI-cut pUC-18 (BR_L), and plasmid isolates of the correct sequence were chosen. The FR4 and c1 constant regions of the humanised anti-CD4 heavy chain were PCR cloned into pUC-18 (BR_L) using oligonucleotide primers X_H (SEQ ID NO: 33) and Y_H (SEQ ID NO: 34). Primer X_H contains SpeI and HindIII sites, and Y_H an EcoRI site. The HindIII and EcoRI sites were used to clone the PCR product into pUC-18, and plasmid isolates of the correct sequence were selected. The complete heavy chain was subsequently reconstituted from the humanised variable region and γ1 constant region clones using the engineered FR4 Spe1 site.

Humanized YTH 655 heavy and light chains were cloned into a eukaryotic expression vector under human cytomegalovirus promoters and expressed transiently in COS cells at 200 ng/ml as determined by IgG ELISA. A stable

cell line expressing humanized YTH 655 heavy and light chains was made by transfecting NSO cells with the same eukaryotic expression vector used for the COS cell transfections. Binding to YTH 655 and a chimeric YTH 655 containing human IgG1 constant region and YTH 655 variable region were shown by FACS analysis to bind an activated T cell line called MF14. Humanized YTH 655 [4 ug/mg] binding to MF14 cells was equivalent to binding of the rat YTH 655 [4 ug/ml] and chimeric YTH 655 (4 ug/ml] as determined by FACS (Weir D.M. 1985 Handbook of Experimental Immunology Vol 1 and 2 4th Ed-Blackwell Scientific Publication, Oxford). The antigen specificity of the humanized monoclonal anitbody, therefore, has been retained. Binding of humanized YTH 655 to MF14 cells was shown to be concentration dependent by FACS analysis.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

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(F) POSTAL CODE (ZIP): CB2 1QP

(ii) TITLE OF INVENTION: Antibody

(iii) NUMBER OF SEQUENCES: 34

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25 (EPO)

(vi) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: GB 91 25979.6
(B) FILING DATE: 06-DEC-1991

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 330 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..330

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
GAT GTT GTG ATG ACA CAA ACT CCA GTC TCC CTG CCT GTC AGC CTT GGA      48
Asp Val Val Met Thr Gln Thr Pro Val Ser Leu Pro Val Ser Leu Gly
 1               5                  10                  15

GGT CAA GCC TCT ATC TCT TGC CGG TCA AGT CAG AGC CTG GTA CAC AGT      96
Gly Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Val His Ser
            20                  25                  30

AAT GGA AAC ACC TAC TTG CAT TGG TAC CTG CAG AAG CCA GGC CAG TCT     144
Asn Gly Asn Thr Tyr Leu His Trp Tyr Leu Gln Lys Pro Gly Gln Ser
        35                  40                  45

CCA CAG CTC CTC ATC TAT CGG GTT TCC AAC AGA TTT TCT GGG GTG CCA     192
Pro Gln Leu Leu Ile Tyr Arg Val Ser Asn Arg Phe Ser Gly Val Pro
    50                  55                  60

GAC AGG TTC AGT GGC AGT GGG TCA GGG ACA GAT TTC ACC CTC AAG ATC     240
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65                  70                  75                  80

AGC AGA GTA GAG CCT GAG GAC TTG GGA GAT TAT TAC TGC TTA CAA AGT     288
Ser Arg Val Glu Pro Glu Asp Leu Gly Asp Tyr Tyr Cys Leu Gln Ser
                85                  90                  95

ACA CAT TTT CCG TAC ACG TTT GGA GCT GGG ACC AAG CTG GAA             330
Thr His Phe Pro Tyr Thr Phe Gly Ala Gly Thr Lys Leu Glu
            100                 105                 110
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 110 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
Asp Val Val Met Thr Gln Thr Pro Val Ser Leu Pro Val Ser Leu Gly
 1               5                  10                  15

Gly Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Val His Ser
            20                  25                  30

Asn Gly Asn Thr Tyr Leu His Trp Tyr Leu Gln Lys Pro Gly Gln Ser
        35                  40                  45

Pro Gln Leu Leu Ile Tyr Arg Val Ser Asn Arg Phe Ser Gly Val Pro
    50                  55                  60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65                  70                  75                  80

Ser Arg Val Glu Pro Glu Asp Leu Gly Asp Tyr Tyr Cys Leu Gln Ser
                85                  90                  95

Thr His Phe Pro Tyr Thr Phe Gly Ala Gly Thr Lys Leu Glu
            100                 105                 110
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 48 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..48

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
CGG TCA AGT CAG AGC CTG GTA CAC AGT AAT GGA AAC ACC TAC TTG CAT      48
Arg Ser Ser Gln Ser Leu Val His Ser Asn Gly Asn Thr Tyr Leu His
 1               5                  10                  15
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Arg Ser Ser Gln Ser Leu Val His Ser Asn Gly Asn Thr Tyr Leu His
 1               5                  10                  15
```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..21

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```
CGG GTT TCC AAC AGA TTT TCT                                      21
Arg Val Ser Asn Arg Phe Ser
 1               5
```

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```
Arg Val Ser Asn Arg Phe Ser
 1               5
```

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..27

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```
TTA CAA AGT ACA CAT TTT CCG TAC ACG                                  27
Leu Gln Ser Thr His Phe Pro Tyr Thr
 1               5
```

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

```
Leu Gln Ser Thr His Phe Pro Tyr Thr
 1               5
```

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 297 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..297

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

```
GGA GGT TTG GTG AAA CCT GGG GCT TCT CTG AAA CTC TCT TGT GTA GCC      48
Gly Gly Leu Val Lys Pro Gly Ala Ser Leu Lys Leu Ser Cys Val Ala
 1               5                  10                  15

TCG GGA TTC ACT TTC AGT GAC TAC TGG ATG AGC TGG GTT CGC CAG ACT      96
Ser Gly Phe Thr Phe Ser Asp Tyr Trp Met Ser Trp Val Arg Gln Thr
            20                  25                  30

CCT GGA AAG ACC ATG GAG TGG ATT GGA GAT ATT AAA TAT GAT GGC AGT     144
Pro Gly Lys Thr Met Glu Trp Ile Gly Asp Ile Lys Tyr Asp Gly Ser
        35                  40                  45

TAC ACA AAC TAT GCA CCA TCC CTA AAG AAT CGA TTC ACA ATC TCC AGA     192
Tyr Thr Asn Tyr Ala Pro Ser Leu Lys Asn Arg Phe Thr Ile Ser Arg
    50                  55                  60

GAC AAT GCC AAG AGC ACC CTG TAC CTG CAG ATG AGC AAT GTG AGA TCT     240
Asp Asn Ala Lys Ser Thr Leu Tyr Leu Gln Met Ser Asn Val Arg Ser
65                  70                  75                  80

GAG GAC ACA GCC ACT TAT TAC TGT ACT AGA GAG GTA CAA CGG AGT TAC     288
Glu Asp Thr Ala Thr Tyr Tyr Cys Thr Arg Glu Val Gln Arg Ser Tyr
                85                  90                  95

TGG GGC CAA                                                        297
Trp Gly Gln
```

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 99 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Gly Gly Leu Val Lys Pro Gly Ala Ser Leu Lys Leu Ser Cys Val Ala
1 5 10 15

Ser Gly Phe Thr Phe Ser Asp Tyr Trp Met Ser Trp Val Arg Gln Thr
20 25 30

Pro Gly Lys Thr Met Glu Trp Ile Gly Asp Ile Lys Tyr Asp Gly Ser
35 40 45

Tyr Thr Asn Tyr Ala Pro Ser Leu Lys Asn Arg Phe Thr Ile Ser Arg
50 55 60

Asp Asn Ala Lys Ser Thr Leu Tyr Leu Gln Met Ser Asn Val Arg Ser
65 70 75 80

Glu Asp Thr Ala Thr Tyr Tyr Cys Thr Arg Glu Val Gln Arg Ser Tyr
85 90 95

Trp Gly Gln

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..15

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

15

GAC TAC TGG ATG AGC
Asp Tyr Trp Met Ser
1 5

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

```
Asp Tyr Trp Met Ser
 1               5
```

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 51 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..51

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

```
GAT ATT AAA TAT GAT GGC AGT TAC ACA AAC TAT GCA CCA TCC CTA AAG      48
Asp Ile Lys Tyr Asp Gly Ser Tyr Thr Asn Tyr Ala Pro Ser Leu Lys
 1               5                  10                  15

AAT                                                                 51
Asn
```

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

```
Asp Ile Lys Tyr Asp Gly Ser Tyr Thr Asn Tyr Ala Pro Ser Leu Lys
 1               5                  10                  15

Asn
```

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..18

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

18

GAG GTA CAA CGG AGT TAC
Glu Val Gln Arg Ser Tyr
1 5

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Glu Val Gln Arg Ser Tyr
1 5

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

GATCAAGCTT CTCTACAGTT ACTGAGCACA 30

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 47 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

CCATTACTGT GTACCAGGCT CTGACTTGAC CGACAGGAGA TGGAGGC 47

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 47 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

TGGTACACAG TAATGGAAAC ACCTACTTGC ATTGGTACCT GCAGAAG 47

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

AGAAAATCTG TTGGAAACCC GATAGATCAG GAGCTG 36

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

CGGGTTTCCA ACAGATTTTC TGGGGTCCCT GACAGG 36

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 42 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

CGTGTACGGA AAATGTGTAC TTTGTAAGCA GTAATAAACC CC 42

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 42 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

TTACAAAGTA CACATTTTCC GTACACGTTC GGCGGAGGGA CC 42

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

GATCAAGCTT CTAACACTCT CCCCTGTTGA 30

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

TGGGATCGAT CAAGCTTTAC AGTTACTGAG C 31

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

GCTCATCCAG TAGTCACTGA AGATGAATCC 30

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

GACTACTGGA TGAGCTGGGT CCGCCAGGCT 30

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 48 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

TTAGTTTGTG TAACTGCCAT CATATTTAAT ATCTGCGACC CACTCCAG 48

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 51 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

GGCAGTTACA CAAACTATGC ACCATCCCTA AAGAATCGAT TCACTATCTC C 51

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

GTAACTCCGT TGTACCTCTC TTGCACAGAA ATA 33

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 48 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

GAGGTACAAC GGAGTTACTG GGGCCAAGGG TCACTAGTCA CAGTCTCC 48

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

TAGAGTCCTG AGGGAATTCG GACAGCCGGG AAGGTG 36

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 48 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

GCTGCTCCTT TTAAGCTTTG GGGTCAAGGC TCACTAGTCA CAGTCTCC 48

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

AAGCTTCCGT CGAATTCATT TACCCGGAGA CAG 33

CLAIMS:

1. A humanised antibody in which the amino acid sequence of the CDRs is derived from the sequence of the CDRs of a monoclonal antibody having the specificity of binding to resting and activated T-cells, inhibiting T-cell proliferation and lysing T-cells from mice transgenic for human CD2 and in which sufficient of the amino acid sequence of each CDR has been retained to provide the same specificity for the humanised antibody.

2. A humanised antibody according to Claim 1, in which the monoclonal antibody is a mouse or rat monoclonal antibody.

3. A humanised antibody in which sufficient of the amino acid sequence of each CDR shown below is provided such that the antibody is capable of binding to a human T-cell antigen:

light chain:	CDR1 (SEQ ID NOS: 3 and 4)
	CDR2 (SEQ ID NOS: 5 and 6)
	CDR3 (SEQ ID NOS: 7 and 8)
heavy chain:	CDR1 (SEQ ID NOS: 11 and 12)
	CDR2 (SEQ ID NOS: 13 and 14)
	CDR3 (SEQ ID NOS: 15 and 16)

4. An antibody according to any of Claims 1 to 3, in which the variable domain framework of the light chain is substantially homologous to the variable domain framework of the protein HSIGKV11.

5. An antibody according to any of Claims 1 to 4, in which the variable domain framework of the heavy chain is substantially homologous to the variable domain framework of the protein KOL.

6. An antibody according to any of Claims 1 to 5, in which the CDRs are the light chain CDRs 1 to 3 and the heavy chain CDRs 1 to 3 specified in Claim 3.

7. A process for the preparation of a humanised antibody as defined in any of Claims 1 to 6, which process comprises maintaining a host transformed with a first expression vector which encodes the light chain of the humanised antibody and with a second expression vector which encodes the heavy chain of the humanised antibody under such conditions that each chain is expressed and isolating the humanised antibody formed by assembly of the thus-expressed chains.

8. A process according to Claim 7, in which the first expression vector and the second expression vector are the same vector.

9. A DNA sequence encoding the light chain or the heavy chain of a humanised antibody as defined in any of Claims 1 to 6.

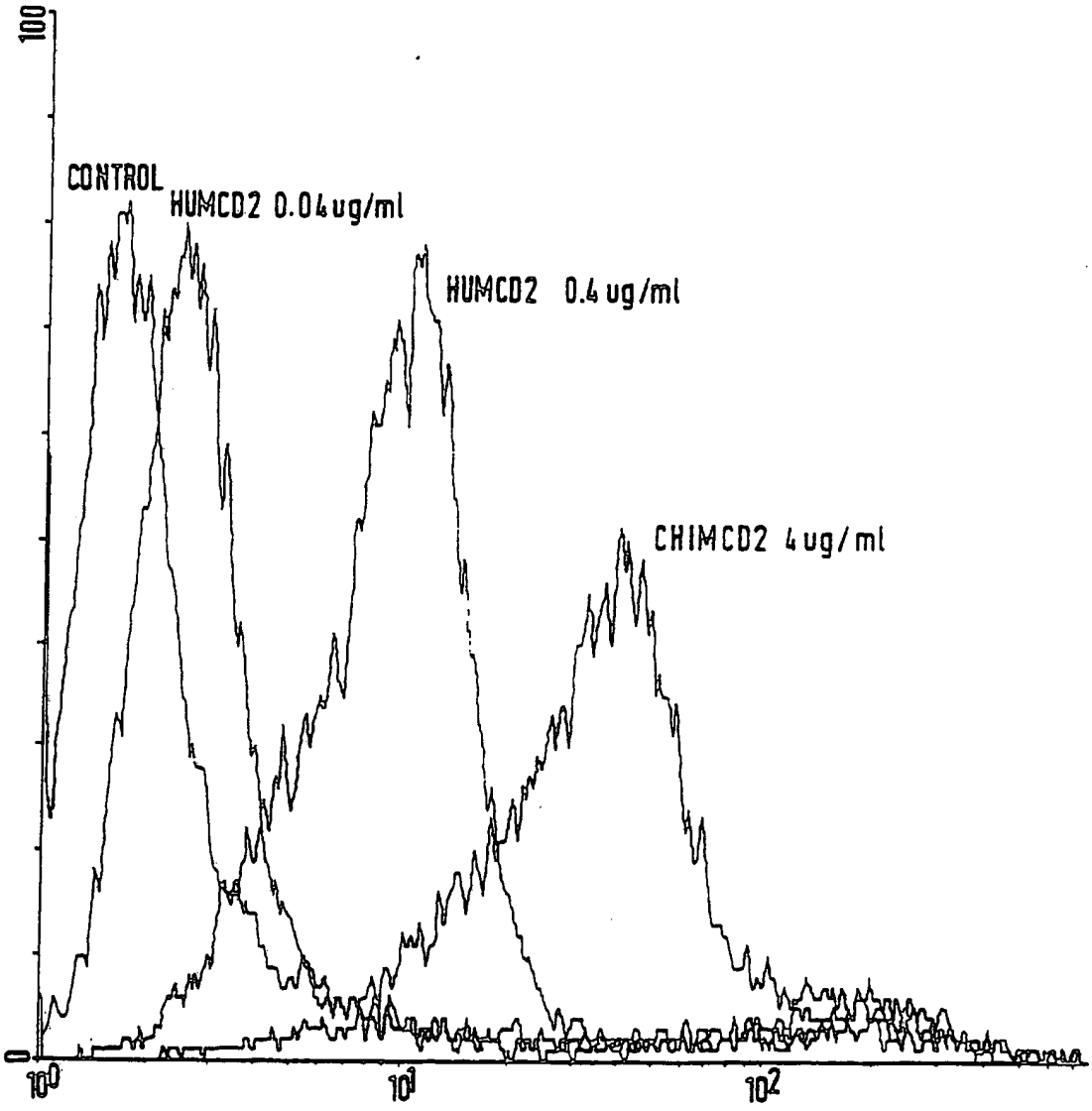
10. An expression vector which incorporates a DNA sequence as claimed in Claim 9.

11. A host transformed with an expression vector as claimed in Claim 10.

12. An immunotoxin comprising a humanised antibody as defined in any of Claims 1 to 6 coupled to a cytotoxic agent.

13. A pharmaceutical composition comprising a pharmaceutically acceptable carrier or diluent and, as active ingredient, a humanised antibody as defined in any of Claims 1 to 6.

14. A composition according to Claim 13, comprising an immunotoxin in which the humanised antibody is coupled to a cytotoxic agent.


100
CONTROL
HUMCD2 0.04ug/ml
HUMCD2 0.4 ug/ml
CHIMCD2 4ug/ml
0
10^0
10^1
10^2

INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 92/02251

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 C12N15/13; C12N15/62; C12P21/08; A61K39/395
C12N5/10; C07K15/28

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	C12N ; C07K ; A61K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category[10]	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
Y	WO,A,9 109 968 (CELLTECH LTD, GB) 11 July 1991 see the whole document ---	1,2,4,5, 7-14
Y	WO,A,9 008 187 (DANA FARBER CANCER INSTITUTE, US) 26 July 1990 see the whole document ---	1,2,4,5, 7-14
Y	WO,A,9 109 967 (CELLTECH LTD, GB) 11 July 1991 see the whole document ---	1,2,4,5, 7-14
Y	PATENT ABSTRACTS OF JAPAN vol. 14, no. 299 (C-0733)27 June 1990 & JP,A,20 97 400 (YAMASA SHOYU CO LTD) 9 April 1990 see abstract ---	1,2,4,5, 7-14

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
04 FEBRUARY 1993	23.
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT FFICE	NAUCHE S.A.

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
A	WO,A,8 809 344 (CREATIVE BIOMOLECULES, INC.) 1 December 1988 ---	
A	JOURNAL OF MOLECULAR BIOLOGY vol. 196, no. 4, 1987, ACADEMIC PRESS pages 901 - 917 Chothia, C.; Lesk, A.M.; 'Canonical structures of the hypervariable regions of Immunoglobulins.' -----	

ANNEX TO THE INTERNATIONAL SEARCH REPORT
ON INTERNATIONAL PATENT APPLICATION NO. GB 9202251
SA 67345

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on
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Patent document cited in search report	Publication date	Patent family member(s)		Publication date
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		EP-A-	0460171	11-12-91
		EP-A-	0460178	11-12-91
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		US-A-	5132405	21-07-92
		US-A-	5091513	25-02-92

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International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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C12N 5/10, 5/12, C12P 21/08

A1

(11) International Publication Number: WO 93/25237

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(30) Priority data:
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(60) Parent Application or Grant
(63) Related by Continuation
US 07/904,074 (CON)
Filed on 15 June 1992 (15.06.92)

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(81) Designated States: AU, CA, JP, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.

(54) Title: CHIMERIC ANTI-CEA ANTIBODY

(57) Abstract

A chimeric murine human antibody, the kappa and gamma genes of which have a murine variable region and a human constant region, are described.

FOR THE PURPOSES OF INFORMATION ONLY

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CHIMERIC ANTI-CEA ANTIBODY

This invention was made with government support under Grant No. CA 43904 awarded by the National Institutes of Health. The government has certain rights in the invention.

FIELD OF THE INVENTION

This invention relates to a chimeric mouse-human antibody to carcinoembryomic antigen (CEA) designated T84.12.

BACKGROUND OF THE INVENTION

CEA is a widespread tumor marker. Its expression can be detected in more than 95% of all human colon cancers. It is a member of the immunoglobulin superfamily and is closely related to NCA and BGP.

Of the various available CEA specific monoclonal antibodies, murine T84.66 antibody shows the highest specificity and affinity for CEA (Wagener, et al., J. Immunology 130:2308-2315 (1985)). It has been used successfully for in vivo tumor imaging in mice and humans. It is well suited for the immunodetection and immunotherapy of human colon cancers.

The in vivo human use of T84.66 is limited by its murine origin resulting in immune response against the heterologous immunoglobulin. Chimeric T84.66 was created by use of recombinant gene technology to lessen the immunogenicity in man (see Neumaier, et al., Cancer Research 50:2128-2134 (1990) and United States Patent 5,081,235). The cloned antibody genes including the immunoglobulin promoter were transfected into SP2/0 myeloma cells by electroporation or CHO cells using lipofection. The expressed chimeric mabs were characterized in different nzyme immunoassays and a western blot.

The sequence of the V-regions of th heavy and light chain genes were determined using the well known Sanger chain termination method.

SUMMARY OF THE INVENTION

Murine T84.12 is another well characterized CEA specific monoclonal of the murine IgG2a isotype. It recognizes the same epitope on CEA as T84.66 but with an affinity constant which is lower by a factor of approximately ten (10). For that reason, T84.12 was selected, pursuant to this invention, to generate mouse-human chimeric antibodies for therapeutic purposes in man.

cDNA clones were humanized (chimerized) by shuffling the human IgG1 heavy or light chain constant domain exons, including the 5'-UT and leader peptide, to the variable regions of the heavy and light chain genes of murine T84.12.

The resulting hybridoma produces significant quantities of chimeric T84.12 anti-CEA antibodies useful for, among other things, human therapeutic purposes.

DETAILED DESCRIPTION OF THE INVENTION

Production of the chimeric anti-CEA antibodies of this invention entails a series of steps including, among others, identification of the amino terminal protein sequences of murine T84.12, determination of the cDNA sequence of mouse light chain and heavy chain clones of T84.12 and of the corresponding amino acid sequences and the chimerization of murine T84.14 cDNA clones. One aspect of the invention entails *in vitro* mutagenesis of a mouse T84.12 light chain clone.

Aminoterminal Sequences of Murine T84.12

Murine T84.12 specific light (L) chain clones L1-L4 and T84.12 heavy chain clones H1-H4 were prepared and sequenced in known manner. All four heavy chain clones showed a 100% V-region homology in

th ir V-region and therefore clon H4 was selected for the sequencing of the IgG2a heavy chain constant regions. The variable domains of light chain clones L2, L3 and L4 were identical. Clone L1 was totally different, apparently representing the endogenous transcript. For the complete characterization of the constant kappa light chain domains and the 3'-untranslated region the light chain clones L1, L4 and the heavy chain clone H4 were selected.

Table I sets forth the amino terminal sequences of the T84.12 light and T84.12 heavy chains. The reported sequences were determined using reduced (DTT) and alkylated (iodoacetic acid) purified monoclonal antibody. The heavy and light chains were separated under reducing conditions on a Sephadex G100 column using 1 M acetic acid as a running buffer. The isolated chains were subjected to amino acid sequencing.

TABLE I

Residue	T84.12 light	T84.12 heavy
1	Asp	Glu
2	Ile	Val
3	Val	Lys
4	Leu	Leu
5	Thr	Val
6	Gln	Glu
7	Ser	Ser
8	Gln	Gly
9	Lys	Gly
10	Phe	Gly
11	Met	Phe
12	Gly	Val
13	Thr	Lys
14	Ser	Pro
15	-	Gly

cDNA Sequ nce of Mouse Light Chain Clone T84.12 L4

The sequence of full size cDNA T84.12 clone was determined (1020 bp) in known manner. This clone contained a very short 5'-UT region of 10 bp which was followed by the ATG start codon. The presence of the entire leader peptide, V-region and the cKappa constant domain could be demonstrated. At the end of the Ckappa constant domain a TAG stop codon was present. The 3'-untranslated region (280 bp) contained a polyadenylation signal (AATAAA) and a poly(A) tail. The entire full size cDNA clone was flanked by the destroyed SmaI restriction cloning site (GGG-CCC). The translation of the obtained nucleotide sequence into the amino acid sequence yielded an open reading frame (bp 34-741 = 708 bp) resulting in 236 amino acids. In addition the Ckappa constant domain showed a 99.7% homology to other published Ckappa constant domain sequences (Kabat). There was only a C to T exchange (see Kabat, et al., "Sequences of Proteins of Immunological Interest", Fourth Ed. U.S. Dept. of Health and Human Services PHS NIH (1987)) in the T84.12 light chain sequence at bp 711 (CATTGT). This base pair difference resulted in a silent mutation. The leader peptide and V-region were different from the T84.66 clones.

In SEQ ID NO. 1, the light chain cDNA sequence of murine T84.12, the following regions are underlined (from the top to the bottom): ATG start codon, start of variable region, start of C-kappa constant domain, TAG stop codon and polyadenylation signal.

Amino Acid Sequence of T84.12 L4 (Frame 1 = 34-741)

In SEQ ID. NO. 2, the light chain amino acid sequence of T84.12, the following regions are underlined (from the top to the bottom): ATG start codon, start of variable region, start of C-kappa constant domain and TAG stop codon.

cDNA Sequence of Mouse H avy Chain Cl ne T84.12 H4

The complete s quence of the full size cDNA clone T84.12 was determined in known manner (1645 bp). This clone contained a 10 bp longer 5'-UT region than the light chain clone L4 which was also followed by the ATG start codon. The presence of the entire leader peptide, V-region and all three constant domain of IgG2a could be demonstrated. At the end of CH3 constant domain of IgG2a a TGA stop codon was present. The 3'-untranslated region (120 bp) contained the polyadenylation signal AATAAA. The entire full size cDNA clone was flanked by the destroyed SmaI restriction cloning site GGG-CCC. The translation of the obtained nucleotide sequence into the amino acid sequence yielded an open reading frame (52-1485 = 1434 bp) resulting in 478 amino acids. In addition, the IgG2a constant domain showed a 98.7% homology to other published IgG2a constant domain sequences (Kabat). The hinge region showed a 100% homology to the Kabat sequence too. Two different codons in the CH1 domain were identical to IgG3 and three different codons in the CH3 domain identical to MOPC21.

In SEQ ID. NO. 3, the heavy chain cDNA sequence of T84.12, the following regions are underlined (from the top to the bottom): ATG start codon, start of variable region, start of CH1 constant domain, start of hinge region, start of CH2 constant domain, start of CH3 constant domain, TGA stop codon and polyadenylation signal.

Amino Acid Sequence of T84.12 H4 (Frame 2 = 52-1485)

In SEQ ID NO. 4, the heavy chain amino acid sequence of T84.12 H4, the following regions are underlined (from the top to the bottom): ATG start

codon, start of variabl region, start of CH1 constant domain, start of hinge region, start of CH2 constant domain, start of CH3 constant domain and TGA stop codon.

Chimeric T84.12

The obtained and characterized full size cDNA murine T84.12 L4 and H4 clones were chimerized using the constant domains of human IgG1 heavy chain cDNAs and the constant domains of human kappa light chain cDNAs respectively. The human heavy and kappa chain constant region sequences were derived from plasmids obtained from Dr. Jeffrey Schlom, National Institutes of Health. The plasmids contained chimeric B72.3 cDNA clones, cloned from cells expressing the chimeric B72.3 antibody (see, Hutzell, et al., Cancer Research 31:181-189 (1991)). Dr. Schlom's group obtained the human gamma and kappa chain genomic expression vectors from Dr. Sherie Morrison, UCLA (Oi, V.T., et al., Biotechniques 4:214 (1986)), in order to make those constructs. Using specific primers, the variable domains of T84.12 (mouse cDNA) were, in known manner, fused in frame to the human constant domain(s) of chimeric B72.3 using the splice overlap extension PCR. See Ho, et al., Gene 77:51-59 (1988) and Horton, et al., Gene 77:61-68 (1989). These full size cDNA's were named CHI T84.12 L3, L6, L8, H2 and H3.

The chimeric clones were used for the production of Fab, $F(ab')_2$-fragments, Fv-fragments and of single chain antibodies linked by a synthetic peptide.

cDNA Sequence of T84.12 L6

The entire sequence of the full size cDNA clone chiT84.12 L6 was determined in known manner (956 bp). The clone chiT84.12 L6 showed the correct

sequence for a mouse-human chimeric T84.12 light chain. The clone chiT84.12 L6 was used for further subcloning into the pHβ-Apr-neo vector (see Gunning, et al., Proc. Natl. Acad. Sci. 84:4831-4835 (1987)) to transfect SP2/0 myeloma cells.

The clone chiT84.12 L6 contained a short 5'-UT region of 9 bp which was followed by the ATG start codon. The presence of the entire leader peptide, V-region and the human Ckappa constant domain could be confirmed. At the end of the human Ckappa constant domain a TAG stop codon was present. The 3'-untranslated region (218 bp) contained a polyadenylation signal (AATAAA). The translation of the obtained nucleotide sequence into the amino acid sequence yielded an open reading frame (bp 34-738 = 705 bp) resulting in 235 amino acids. In addition the human Ckappa constant domain showed a 100% homology to other published Ckappa constant domain sequences (Kabat).

In SEQ ID NO. 7, the light chain cDNA sequence of chiT84.12 L6, the following regions are underlined (from the top to the bottom): ATG start codon, start of mouse variable region, start of human C-kappa constant domain, TAG stop codon and polyadenylation signal.

Coding Sequence of chiT84.12 L6 (bp = 34-738)

In Seq. ID No. 8, the light chain amino acid sequence of chiT84.12 L6, the following regions are underlined (from the top to the bottom): ATG start codon, start of mouse variable region, start of human C-kappa constant domain and TAG stop codon.

cDNA Sequence of Chimeric T84.12 H3

The complete sequence of the full size cDNA cl ne chiT84.12 H3 was determined in known manner (1641 bp). The clone chiT84.12 H3 showed the correct sequence for a mouse-human chimeric T84.12 heavy chain and had one mutation at the beginning of the CH2 domain (GTG to GCG at position 484 = valine against alanine) and one at the end of the 3'-UT (AAATAAA to GAATAAA). However, this did not affect the polyadenylation signal. The clone chiT84.12 H3 was used for further subcloning into the pHβ-Apr-gpt vector to transfect SP2/0 myeloma cells which are expressing chiT84.12 kappa light chains.

This clone contained a 41 bp long 5'-UT region which was followed by the ATG start codon. The presence of the entire leader peptide, mouse V-region and all three human constant domain of IgG1 could be demonstrated. At the end of CH3 constant domain of IgG1 a TGA stop codon was present. The 3'-untranslated region (153 bp) contained the polyadenylation signal AATAAA. The translation of the obtained nucleotide sequence into the amino acid sequence yielded an open reading frame (bp 52-1485 = 1410 bp) resulting in 470 amino acids. In addition, the human IgG1 constant domain showed a 100% homology to other published IgG1 constant domain sequences (Kabat). The hinge region showed a 100% homology to the Kabat sequence too.

In SEQ ID NO. 9, the heavy chain cDNA sequence of chiT84.12 H3, the following regions are underlined (from the top to the bottom): ATG start codon, start of mouse variable region, start of human CH1 constant domain, start of hinge region, start of CH2 constant domain, start of CH3 constant domain, TGA stop codon and polyadenylation signal.

In SEQ ID NO. 10, the h avy chain amino acid sequenc of chiT84.12 H3, the following regions are underlined (from the top to the bottom): ATG start codon, start of mouse variable region, start of human CH1 constant domain, start of hinge region, start of CH2 constant domain, start of CH3 constant domain and TGA stop codon.

In Vitro Mutagenesis of Mouse T84.12 L4 cDNA

With some exceptions, two cysteine residues are typically present in an immunoglobulin domain. The CDR3 (L3) of T84.12 light chain clone L4 contained an additional third cysteine residue in the mouse variable kappa light chain domain. The presence of the third cysteine is apparently related to the loss of binding activity by murine T84.12 after dissociation of both chains and chemical crosslinking using homobifunctional crosslinking agents. Therefore the cysteine (TGT) in position 364-366, see SEQ ID NO. 1, (amino acid residue 91) was changed to a serine (TCT) by site directed mutagenesis.

Overview of MUTA-GENE Phagemid In Vitro Mutagenesis

The mutagenesis was carried out using the MUTA-GENE phagemid in vitro mutagenesis kit from BioRad. The original procedure was simplified and reduced to the following eleven steps:

1. Subcloning of the coding cDNA strand in pTZ18U or pTZ19U phagemids (depending on the orientation of cloned cDNA in pUC18).

2. Electrotransformation of E. coli CJ236 with pTZ18U or 19U containing the cDNA to be mutagenized (plate on LB-amp + 30 μg/ml chloramphenicol).

3. Miniprep DNA isolation from single recombinant CJ236 colonies. This E. coli strain incorporates uracil residues into the phagemid DNA.

4. Growth of uracil containing phagemids in 2xYT media (containing ampicillin (50 μg/ml) and chloramphenicol (30 μg/ml). Start out with a marked and mini prep DNA analyzed single colony from the plate. Add the helper phage M13K07 in order to obtain single stranded phagemid DNA.

5. PEG extraction and purification (PCI) of single stranded phagemid DNA.

6. Phosphorylation of the mutagenesis primer (represents the minus strand and binds to the single stranded plus strand phagemid DNA).

7. Synthesis of the mutagenic strand by annealing of the phosphorylated mutagenesis primer to the purified single stranded phagemid DNA. The complementary minus strand is created by the T4 DNA polymerase and gaps sealed with the T4DNA ligase.

8. Electrotransformation of E. coli MV1190 with double-stranded mutagenized cDNA. This strain removes uracil residues.

9. Isolation of miniprep DNA from single growing recombinant MV1190 colonies. The insert size can be determined by restriction enzyme digest and compared to the wild type.

10. Sequence several miniprep DNA from the mutants and compare it with the wild type sequence.

11. Select clones with the correct mutations and grow a larger culture (100 ml). Purify the mutagenized cDNA using Qiagen columns and confirm the entire sequence of the mutated cDNA clone.

One such clone, named T84.12 L4-12-1 was selected for exemplification of the invention.

cDNA S qu nce of T84.12 L4-12-1

The entire sequ nce of the full size cDNA clon T84.12 L4-12-1 was determined in known manner (1999 bp). The clone showed the correct sequence for a mouse T84.12 light chain and the introduced cysteine to serine mutation. It was used for further subcloning into the PHβ-Apr-neo vector (See Gunning, et al., Proc. Natl. Acad. Sci. 84:4831-4835 (1987)) to transfect SP2/0 myeloma cells.

This T84.12 L4-12-1 clone contained a very short 5'-UT region of 10 bp which was followed by the ATG start codon. The presence of the entire leader peptide, V-region and the Ckappa constant domain could be demonstrated. At the end of the Ckappa constant domain a TAG stop codon was present. The 3'-untranslated region (280 bp) contained a polyadenylation signal (AATAAA). The entire full size cDNA clone was flanked by the destroyed SmaI restriction cloning site (GGG-CCC). The translation of the obtained nucleotide sequence into the amino acid sequence yielded an open reading frame (bp 34-741 = 708 bp) resulting in 236 amino acids. In addition, the Ckappa constant domain showed a 99.7% homology to other published cKappa constant domain sequences (Kabat). There was only a C (Kabat) to T exchange in the T84.12 light chain sequence at bp 711 (CATTGT). This base pair difference resulted in a silent mutation.

In SEQ ID NO. 5, the light chain cDNA sequence of T84.12 L4-12-1, the following regions were underlined (from the top to the bottom): ATG start codon, start of mouse variable region, start of human C-kappa constant domain, TAG stop codon and polyadenylation signal. The mutagenized TGT (cys) to TCT (ser) is underlined and in italics.

Amino Acid Sequence of T84.12 L4-12-1

(Frame 1 = 34-741)

In SEQ ID NO. 6, the light chain amino acid sequence of T84.12, the following regions are underlined (from the top to the bottom): ATG start codon, start of variable region, start of C-kappa constant domain and TAG stop codon. The mutagenized TGT (cys) to TCT (ser) is underlined and in italics. All other cysteine residues are underlined.

Expression of Mutagenized Mouse T84.12 cDNAs

The mutated light chain (T84.12 L4-1) cDNA and the normal heavy chain (T84.12 H4) cDNA were transferred in a β-actin cDNA expression vector (Gunning, et al., supra) and cotransformed into Sp2/0 myeloma cells by electroporation. The vectors include the human β-actin promoter, intervening sequence, cloning site, and a polyadenylation signal. Since the vectors contain the neomycin-resistance gene, transfectants were selected in the presence of the drug, G418. Clones were expanded and evaluated for antibody production (kappa or gamma chain) and CEA-binding activity by ELISAs. Although levels of expression were low, there was a correlation between antibody and anti-CEA activity in culture supernatants.

Binding Activity of T84.12 cys --> Ser Mutant

Clone	Kappa chain (ng/ml)	Gamma chain (ng/ml)	Anti-CEA activity (ng/ml)
4C1	2-6	2-6	2
4H9	6-18	6-18	3-8
1B1	6-18	2-6	1
4A3	6-18	2-6	1-3
5A11	2-6	2-6	1-3

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: John E. Shively
Rainer Fischer
Anna Wu
Ray Paxton
Y.H. Joy Yang

(ii) TITLE OF INVENTION: Chimeric Anti-CEA Antibody

(iii) NUMBER OF SEQUENCES: 10

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: City of Hope

(B) STREET: 1500 East Duarte Road

(C) CITY: Duarte

(D) STATE: California

(E) COUNTRY: United States of America

(F) ZIP: 91010-0269

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: 3M Double Density 5 1/4" diskette

(B) COMPUTER: Wang PC

(C) OPERATING SYSTEM: MS-DOS (R) Version 3.30

(D) SOFTWARE: Microsoft (R)

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER: 07/904,074

(B) FILING DATE: 15 June 1992

(C) CLASSIFICATION: Unknown

(vii) PRIOR APPLICATION DATA: None

(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: Irons, Edward S.

(B) REGISTRATION NUMBER: 16,541

(C) REFERENCE/DOCKET NUMBER: None

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(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1041

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single Stranded

(D) TOPOLOGY: Unknown

(ii) MOLECULE TYPE: Nucleic Acid

(iii) HYPOTHETICAL: Not Applicable

(iv) ANTI-SENSE: Not Applicable

(v) FRAGMENT TYPE: Not Applicable

(vi) ORIGINAL SOURCE: Synthically Prepared

(vii) IMMEDIATE SOURCE: Synthetically Prepared

(viii) POSITION IN GENOME: None

(ix) FEATURE: None

(x) PUBLICATION INFORMATION: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

```
TTACGAATTC GAGCTCGGTA CCCGGGCATC AAGATGGAGT CACAGACTCA      50
GGTCTTTGTA TACATGTTGC TGTGGTTGTC TGGTGTTGAT GGAGACATTG     100
TGCTGACCCA GTCTCAAAAA TTCATGTCCA CATCAGTTGG AGGCACGGTC     150
AGCGTCACCT GCAAGGCCAG TCAAAATGTG CATACTAATG TTGCCTGGTA     200
TCAACAGAAA CCAGGACAAT CTCCTAAAGC ACTGATTTAC TCGGCATCCT     250
ACCGTTACAG TGGAGTCCCT GATCGCTTCA CAGGCAGTGG ATCTGGGACA     300
GATTTCACTC TCACCATCAG CAATGTGCAG TCTGAAGACT TGGCAGAATA     350
TTTCTGTCAG CAATGTAACA GCTATCCTCT ATTCACGTTC GGCTCGGGGA     400
CAACGTTGGA AATAAAACGG GCTGATGCTG CACCAACTGT ATCCATCTTC     450
CCACCATCCA GTGAGCAGTT AACATCTGGA GGTGCCTCAG TCGTGTGCTT     500
CTTGAACAAC TTCTACCCCA AAGACATCAA TGTCAAGTGG AAGATTGATG     550
GCAGTGAACG ACAAAATGGC GTCCTGAACA GTTGGACTGA TCAGGACAGC     600
AAAGACAGCA CCTACAGCAT GAGCAGCACC CTCACGTTGA CCAAGGACGA     650
GTATGAACGA CATAACAGCT ATACCTGTGA GGCCACTCAC AAGACATCAA     700
CTTCACCCAT TGTCAAGAGC TTCAACAGGA ATGAGTGTTA GAGACAAAGG     750
TCCTGAGACG CCACCACCAG CTCCCCAGCT CCATCCTATC TTCCCTTCTA     800
AGGTCTTGGA GGCTTCCCCA CAAGCGACCT ACCACTGTTG CGGTGCTCCA     850
AACCTCCTCC CCACCTCCTT CTCCTCCTCC TCCCTTTCCT TGGCTTTTAT     900
CATGCTAATA TTTGCAGAAA ATATTCAATA AAGTGAGTCT TTGCACTTGA     950
AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA    1000
AAAAAAAAAA AAGGGGATCC TCTAGAGTCG ACCTGCAGGC A             1041
```

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 235

(B) TYPE: Amino Acid

(C) STRANDEDNESS: Single Stranded

(D) TOPOLOGY: Unknown

(ii) MOLECULE TYPE: Amino Acid

(iii) HYPOTHETICAL: Not Applicable

(iv) ANTI-SENSE: Not Applicable

(v) FRAGMENT TYPE: Not Applicable

(vi) ORIGINAL SOURCE: Synthetically Prepared

(vii) IMMEDIATE SOURCE: Syntehtically Prepared

(viii) POSITION IN GENOME: None

(ix) FEATURE: None

(x) PUBLICATION INFORMATION: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

```
Met Glu S r Gln Thr Gln Val Phe Val Tyr Met Leu Leu Trp Leu
 1               5                  10                  15
Ser Gly Val Asp Gly Asp Ile Val Leu Thr Gln Ser Gln Lys Phe
            20                  25                  30
Met Ser Thr Ser Val Gly Gly Thr Val Ser Val Thr Cys Lys Ala
        35                  40                  45
Ser Gln Asn Val His Thr Asn Val Ala Trp Tyr Gln Gln Lys Pro
    50                  55                  60
Gly Gln Ser Pro Lys Ala Leu Ile Tyr Ser Ala Ser Tyr Arg Tyr
65                  70                  75
Ser Gly Val Pro Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr Asp
            80                  85                  90
Phe Thr Leu Thr Ile Ser Asn Val Gln Ser Glu Asp Leu Ala Glu
        95                  100                 105
Tyr Phe Cys Gln Gln Cys Asn Ser Tyr Pro Leu Phe Thr Phe Gly
    110                 115                 120
Ser Gly Thr Thr Leu Glu Ile Lys Arg Ala Asp Ala Ala Pro Thr
125                 130                 135
Val Ser Ile Phe Pro Pro Ser Ser Glu Gln Leu Thr Ser Gly Gly
            140                 145                 150
Ala Ser Val Val Cys Phe Leu Asn Asn Phe Tyr Pro Lys Asp Ile
        155                 160                 165
Asn Val Lys Trp Lys Ile Asp Gly Ser Glu Arg Gln Asn Gly Val
    170                 175                 180
Leu Asn Ser Trp Thr Asp Gln Asp Ser Lys Asp Ser Thr Tyr Ser
185                 190                 195
MET Ser Ser Thr Leu Thr Leu Thr Lys Asp Glu Tyr Glu Arg His
            200                 205                 210
Asn Ser Tyr Thr Cys Glu Ala Thr His Lys Thr Ser Thr Ser Pro
        215                 220                 225
Ile Val Lys Ser Phe Asn Arg Asn Glu Cys
    230                 235
```

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1645

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single Stranded

(D) TOPOLOGY: Unknown

(ii) MOLECULE TYPE: Nucleic Acid

(iii) HYPOTHETICAL: Not Applicable

(iv) ANTI-SENSE: Not Applicable

(v) FRAGMENT TYPE: Not Applicable

(vi) ORIGINAL SOURCE: Synthetically Prepared

(vii) IMMEDIATE SOURCE: Synthetically Prepared

(viii) POSITION IN GENOME: None

(ix) FEATURE: None

(x) PUBLICATION INFORMATION: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

```
TTACGAATTC GAGCTCGGTA CCCCCTGGAT TTGAGTTCCT CACATTCAGT      50
CATGAGCACT GAACACAGAC ACCTCACCAT GAACTTCGGG TTCAGCCTGA     100
TTTTCCTTGT CCTTGTTTTA AAAGGTGTCC AGTGTGAAGT GAAGCTGGTG     150
GAGTCTGGGG GAGGCTTTGT GAAGCCTGGA GGGTCCCTGA AACTCTCCTG     200
TGCAGCCTCC GGATTCACTT TCAGTAGTTA TGCCATGTCT TGGGTTCGCC     250
AGACTCCAGA GAAGAGGCTG GAGTGGGTCG CATCCATTAG TAGTGATGGT     300
ATCACCTTCT ATGTAGACAG TGTGAAGGGC CGATTCACCG TCTCCAGAGA     350
CAATGCCAGG AACATCCTGT ACCTGCAAAT GAGCAGTCTG AGGTCTGAGG     400
ACACGGCCAT GTATTACTGT GCAAGAATCG ACTACTACGG AGGAGGGGGA     450
TTTGGTTACT GGGGCCAAGG GACTCTGGCC ACTGTCTCTG CAGCCAAAAC     500
AACAGCCCCA TCGGTCTATC CACTGGCCCC TGTGTGTGGA GATACAACTG     550
GCTCCTCGGT GACTCTAGGA TGCCTGGTCA AGGGTTATTT CCCTGAGCCA     600
GTGACCTTGA CCTGGAACTC TGGATCCCTG TCCAGTGGTG TGCACACCTT     650
CCCAGCTGTC CTGCAGTCTG ACCTCTACAC CCTCAGCAGC TCAGTGACTG     700
TAACCTCGAG CACCTGGCCC AGCCAGTCCA TCACCTGCAA TGTGGCCCAC     750
CCGGCAAGCA GCACCAAGGT GGACAAGAAA ATTGAGCCCA GAGGGCCCAC     800
AATCAAGCCC TGTCCTCCAT GCAAATGCCC AGCACCTAAC CTCTTGGGTG     850
GACCATCCGT CTTCATCTTC CCTCCAAAGA TCAAGGATGT ACTCATGATC     900
TCCCTGAGCC CCATAGTCAC ATGTGTGGTG GTGGATGTGA GCGAGGATGA     950
CCCAGATGTC CAGATCAGCT GGTTTGTGAA CAACGTGGAA GTACACACAG    1000
CTCAGACACA AACCCATAGA GAGGATTACA ACAGTACTCT CCGGGTGGTC    1050
AGTGCCCTCC CCATCCAGCA CCAGGACTGG ATGAGTGGCA AGGAGTTCAA    1100
ATGCAAGGTC AACAACAAAG ACCTCCCAGC GCCCATCGAG AGAACCATCT    1150
CAAAACCCAA AGGGTCAGTA AGAGCTCCAC AGGTATATGT CTTGCCTCCA    1200
CCAGAAGAAG AGATGACTAA GAAACAGGTC ACTCTGACCT GCATGGTCAC    1250
AGACTTCATG CCTGAAGACA TTTACGTGGA GTGGACCAAC AACGGGAAAA    1300
CAGAGCTAAA CTACAAGAAC ACTGAACCAG TCCTGGACTC TGATGGTTCT    1350
TACTTCATGT ACAGCAAGCT GAGAGTGGAA AAGAAGAACT GGGTGGAAAG    1400
AAATAGCTAC TCCTGTTCAG TGGTCCACGA GGGTCTGCAC AATTACCACA    1450
CGACTAAGAG CTTCTCCCGG ACTCCGGGTA AATGAGCTCA GCACCCACAA    1500
AACTCTCAGG TCCAAAGAGA CACCCACACT CATCTCCATG CTTCCCTTGT    1550
ATAAATAAAG CACCCAGCAA TGCCTGGGAC CATGTAAAAA AAAAAAAAAA    1600
AAAAAAAAAA AAAAAAGGGG ATCCTCTAGA GTCGACCTGC AGGCA         1645
```

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 477

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single Stranded

(D) TOPOLOGY: Unknown

(ii) MOLECULE TYPE: Nucleic Acid

(iii) HYPOTHETICAL: Not Applicable

(iv) ANTI-SENSE: Not Applicable

(v) FRAGMENT TYPE: Not Applicable

(vi) ORIGINAL SOURCE: Synthetically Prepared

(vii) IMMEDIATE SOURCE: Synthetically Prepared

(viii) POSITION IN GENOME: None

(ix) FEATURE: None

(x) PUBLICATION INFORMATION: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

```
MSTNHRHNTM NNGNSNNNNV NVNKGVNCNV KNVNSGGGNV KNGGSNKNSC     50
AASGNTNSSY AMSWVRNTNN KRNNWVASNS SDGNTNYVDS VKGRNTVSRD    100
NARNNNYNNM SSNRSNDTAM YYCARNDYYG GGGNGYWGNG TNATVSAAKT    150
TANSVYNNAN VCGDTTGSSV TNGCNVKGYN NNNVTNTWNS GSNSSGVHTN    200
NAVNNSDNYT NSSSVTVTSS TWNSNSNTCN VAHNASSTKV DKKNNNRGNT    250
NKNCNNCKCN ANNNNGGNSV NNNNNKNKDV NMNSNSNNVT CVVVDVSNDD    300
NDVNNSWNVN NVNVHTANTN THRNDYNSTN RVVSANNNNH NDWMSGKNNK    350
CKVNNKDNNA NNNRTNSKNK GSVRANNVYV NNNNNNNMTK KNVTNTCMVT    400
DNMNNDNYVN WTNNGKTNNN YKNTNNVNDS DGSYNMYSKN RVNKKNWVNR    450
NSYSCSVVHN GNHNYHTTKS NSRTNGK                              477
```

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1041

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single Stranded

(D) TOPOLOGY: Unknown

(ii) MOLECULE TYPE: Nucleic Acid

(iii) HYPOTHETICAL: Not Applicable

(iv) ANTI-SENSE: Not Applicable

(v) FRAGMENT TYPE: Not Applicable

(vi) ORIGINAL SOURCE: Synthetically Pr pared

(vii) IMMEDIATE SOURCE: Synthetically Prepared

(viii) POSITION IN GENOME: None

(ix) FEATURE: None

(x) PUBLICATION INFORMATION: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

```
TTACGAATTC GAGCTCGGTA CCCGGGCATC AAGATGGAGT CACAGACTCA      50
GGTCTTTGTA TACATGTTGC TGTGGTTGTC TGGTGTTGAT GGAGACATTG     100
TGCTGACCCA GTCTCAAAAA TTCATGTCCA CATCAGTTGG AGGCACGGTC     150
AGCGTCACCT GCAAGGCCAG TCAAAATGTG CATACTAATG TTGCCTGGTA     200
TCAACAGAAA CCAGGACAAT CTCCTAAAGC ACTGATTTAC TCGGCATCCT     250
ACCGTTACAG TGGAGTCCCT GATCGCTTCA CAGGCAGTGG ATCTGGGACA     300
GATTTCACTC TCACCATCAG CAATGTGCAG TCTGAAGACT TGGCAGAATA     350
TTTCTGTCAG CAATGTAACA GCTATCCTCT ATTCACGTTC GGCTCGGGGA     400
CAACGTTGGA AATAAAACGG GCTGATGCTG CACCAACTGT ATCCATCTTC     450
CCACCATCCA GTGAGCAGTT AACATCTGGA GGTGCCTCAG TCGTGTGCTT     500
CTTGAACAAC TTCTACCCCA AAGACATCAA TGTCAAGTGG AAGATTGATG     550
GCAGTGAACG ACAAAATGGC GTCCTGAACA GTTGGACTGA TCAGGACAGC     600
AAAGACAGCA CCTACAGCAT GAGCAGCACC CTCACGTTGA CCAAGGACGA     650
GTATGAACGA CATAACAGCT ATACCTGTGA GGCCACTCAC AAGACATCAA     700
CTTCACCCAT TGTCAAGAGC TTCAACAGGA ATGAGTGTTA GAGACAAAGG     750
TCCTGAGACG CCACCACCAG CTCCCCAGCT CCATCCTATC TTCCCTTCTA     800
AGGTCTTGGA GGCTTCCCCA CAAGCGACCT ACCACTGTTG CGGTGCTCCA     850
AACCTCCTCC CCACCTCCTT CTCCTCCTCC TCCCTTTCCT TGGCTTTTAT     900
CATGCTAATA TTTGCAGAAA ATATTCAATA AAGTGAGTCT TTGCACTTGA     950
AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA    1000
AAAAAAAAAA AAGGGGATCC TCTAGAGTCG ACCTGCAGGC A             1041
```

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 235

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single Stranded

(D) TOPOLOGY: Unknown

(ii) MOLECULE TYPE: Nucleic Acid

(iii) HYPOTHETICAL: Not Applicable

(iv) ANTI-SENSE: Not Applicable

(v) FRAGMENT TYPE: Not Applicable

(vi) ORIGINAL SOURCE: Synthetically Pr pared

(vii) IMMEDIATE SOURCE: Synthetically Prepared

(viii) POSITION IN GENOME: None

(ix) FEATURE: None

(x) PUBLICATION INFORMATION: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

```
MNSNTNVNVY MNNWNSGVDG DNVNTNSNKN MSTSVGGTVS VTCKASNNVH     50
TNVAWYNNKN GNSNKANNYS ASYRYSGVND RNTGSGSGTD NTNTNSNVNS    100
NDNANYNCNN SNSYNNNTNG SGTTNNNKRA DAANTVSNNN NSSNNNTSGG    150
ASVVCNNNNN YNKDNNVKWK NDGSNRNNGV NNSWTDNDSK DSTYSMSSTN    200
TNTKDNYNRH NSYTCNATHK TSTSNNVKSN NRNNC                    235
```

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 957

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single Stranded

(D) TOPOLOGY: Unknown

(ii) MOLECULE TYPE: Nucleic Acid

(iii) HYPOTHETICAL: Not Applicable

(iv) ANTI-SENSE: Not Applicable

(v) FRAGMENT TYPE: Not Applicable

(vi) ORIGINAL SOURCE: Synthetically Prepared

(vii) IMMEDIATE SOURCE: Synthetically Prepared

(viii) POSITION IN GENOME: None

(ix) FEATURE: None

(x) PUBLICATION INFORMATION: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

```
TTACGAATTC GAGCTCGGTA CCCGGGCATC AAGATGGAGT CACAGACTCA      50
GGTCTTTGTA TACATGTTGC TGTGGTTGTC TGGTGTTGAT GGAGACATTG     100
TGCTGACCCA GTCTCAAAAA TTCATGTCCA CATCAGTTGG AGGCACGGTC     150
AGCGTCACCT GCAAGGCCAG TCAAAATGTG CATACTAATG TTGCCTGGTA     200
TCAACAGAAA CCAGGACAAT CTCCTAAAGC ACTGATTTAC TCGGCATCCT     250
ACCGTTACAG TGGAGTCCCT GATCGCTTCA CAGGCAGTGG ATCTGGGACA     300
GATTTCACTC TCACCATCAG CAATGTGCAG TCTGAAGACT TGGCAGAATA     350
TTTCTGTCAG CAATGTAACA GCTATCCTCT ATTCACGTTC GGCTCGGGGA     400
CAACGTTGGA AATAAAAACT GTGGCTGCAC CATCTGTCTT CATCTTCCCG     450
CCATCTGATG AGCAGTTGAA ATCTGGAACT GCCTCTGTTG TGTGCCTGCT     500
GAATAACTTC TATCCCAGAG AGGCCAAAGT ACAGTGGAAG GTGGATAACG     550
CCCTCCAATC GGGTAACTCC CAGGAGAGTG TCACAGAGCA GGACAGCAAG     600
GACAGCACCT ACAGCCTCAG CAGCACCCTG ACGCTGAGCA AAGCAGACTA     650
CGAGAAACAC AAAGTCTACG CCTGCGAAGT CACCCATCAG GGCCTGAGCT     700
CGCCCGTCAC AAAGAGCTTC AACAGGGGAG AGTGTTAGAG GGAGAAGTGC     750
CCCCACCTGC TCCTCAGTTC CAGCCTGACC CCCTCCCATC CTTTGGCCTC     800
TGACCCTTTT TCCACAGGGG ACCTACCCCT ATTGCGGTCC TCCAGCTCAT     850
CTTTCACCTC ACCCCCCTCC TCCTCCTTGG CTTTAATTAT GCTAATGTTG     900
GAGGAGAATG AATAAATAAA GTGAATCTTT GCAAAAAGCT TGGCACTGGC     950
CGTCGTT                                                   957
```

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 234

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single Stranded

(D) TOPOLOGY: Unknown

(ii) MOLECULE TYPE: Nucleic Acid

(iii) HYPOTHETICAL: Not Applicable

(iv) ANTI-SENSE: Not Applicable

(v) FRAGMENT TYPE: Not Applicable

(vi) ORIGINAL SOURCE: Synthetically Prepared

(vii) IMMEDIATE SOURCE: Synthetically Prepared

(viii) POSITION IN GENOME: None

(ix) FEATURE: None

(x) PUBLICATION INFORMATION: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

```
MNSNTNVNVY MNNWNSGVDG DNVNTNSNKN MSTSVGGTVS VTCKASNNVH      50
TNVAWYNNKN GNSNKANNYS ASYRYSGVND RNTGSGSGTD NTNTNSNVNS     100
NDNANYNCNN CNSYNNNTNG SGTTNNNKTV AANSVNNNNN SDNNNKSGTA     150
SVVCNNNNNY NRNAKVNWKV DNANNSGNSN NSVTNNDSKD STYSNSSTNT     200
NSKADYNKHK VYACNVTHNG NSSNVTKSNN RGNC                      234
```

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1641

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single Stranded

(D) TOPOLOGY: Unknown

(ii) MOLECULE TYPE: Nucleic Acid

(iii) HYPOTHETICAL: Not Applicable

(iv) ANTI-SENSE: Not Applicable

(v) FRAGMENT TYPE: Not Applicable

(vi) ORIGINAL SOURCE: Synthetically Prepared

(vii) IMMEDIATE SOURCE: Synthetically Prepared

(viii) POSITION IN GENOME: None

(ix) FEATURE: None

(x) PUBLICATION INFORMATION: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

```
TTACGAATTC GAGCTCGGTA CCCCCTGGAT TTGAGTTCCT CACATTCAGT      50
GATGAGCACT GAACACAGAC ACCTCACCAT GAACTTCGGG TTCAGCCTGA     100
TTTTCCTTGT CCTTGTTTTA AAAGGTGTCC AGTGTGAAGT GAAGCTGGTC     150
GAGTCTGGGG GAGGCTTTGT GAAGCCTGGA GGGTCCCTGA AACTCTCCTG     200
TGCAGCCTCC GGATTCACTT TCAGTAGTTA TGCCATGTCT TGGGTTCGCC     250
AGACTCCAGA GAAGAGGCTG GAGTGGGTCG CATCCATTAG TAGTGATGGT     300
ATCACCTTCT ATGTAGACAG TGTGAAGGGC CGATTCACCG TCTCCAGAGA     350
CAATGCCAGG AACATCCTGT ACCTGCAAAT GAGCAGTCTG AGGTCTGAGG     400
ACACGGCCAT GTATTACTGT GCAAGAATCG ACTACTACGG AGGAGGGGGA     450
TTTGGTTACT GGGGCCAAGG GACTCTGGCC ACTGTCTCTG CAGCCTCCAC     500
CAAGGGCCCA TCGGTCTTCC CCCTGGCACC CTCCTCCAAG AGCACCTCTG     550
GGGGCACAGC GGCCCTGGGC TGCCTGGTCA AGGACTACTT CCCCGAACCG     600
GTGACGGTGT CGTGGAACTC AGGCGCCCTG ACCAGCGGCG TGCACACCTT     650
CCCGGCTGTC CTACAGTCCT CAGGACTCTA CTCCCTCAGC AGCGTGGTGA     700
CCGTGCCCTC CAGCAGCTTG GGCACCCAGA CCTACATCTG CAACGTGAAT     750
```

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CACAAGCCCA GCAACACCAA GGTGGACAAG AAAGTTGAGC CCAAATCTTG     800
TGACAAAACT CACACATGCC CACCGTGCCC AGCACCTGAA CTCCTGGGGG     850
GACCGTCAGT CTTCCTCTTC CCCCCAAAAC CCAAGGACAC CCTCATGATC     900
TCCCGGACCC CTGAGGTCAC ATGCGTGGTG GTGGACGCGA GCCACGAAGA     950
CCCTGAGGTC AAGTTCAACT GGTACGTGGA CGGCGTGGAG GTGCATAATG    1000
CCAAGACAAA GCCGCGGGAG GAGCAGTACA ACAGCACGTA CCGTGTGGTC    1050
AGCGTCCTCA CCGTCCTGCA CCAGGACTGG CTGAATGGCA AGGAGTACAA    1100
GTGCAAGGTC TCCAACAAAG CCCTCCCAGC CCCCATCGAG AAAACCATCT    1150
CCAAAGCCAA AGGGCAGCCC CGAGAACCAC AGGTGTACAC CCTGCCCCCA    1200
TCCCGGGATG AGCTGACCAA GAACCAGGTC AGCCTGACCT GCCTGGTCAA    1250
AGGCTTCTAT CCCAGCGACA TCGCCGTGGA GTGGGAGAGC AATGGGCAGC    1300
CGGAGAACAA CTACAAGACC ACGCCTCCCG TGCTGGACTC CGACGGCTCC    1350
TTCTTCCTCT ACAGCAAGCT CACCGTGGAC AAGAGCAGGT GGCAGCAGGG    1400
GAACGTCTTC TCATGCTCCG TGATGCATGA GGCTCTGCAC AACCACTACA    1450
CGCAGAAGAG CCTCTCCCTG TCTCCGGGTA AATGAGTGCG ACGGCCGGCA    1500
AGCCCCCGCT CCCCGGGCTC TCGCGGTCGC ACGAGGATGC TTGGCACGTA    1550
CCCCCTGTAC ATACTTCCCG GGCGCCCAGC ATGGGAATAA AGCACCCAGC    1600
GCTGCCCTGG GCCCCTGCAA GGATCCAAGC TTGGCACTGG C             1641
```

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 477

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single Stranded

(D) TOPOLOGY: Unknown

(ii) MOLECULE TYPE: Nucleic Acid

(iii) HYPOTHETICAL: Not Applicable

(iv) ANTI-SENSE: Not Applicable

(v) FRAGMENT TYPE: Not Applicable

(vi) ORIGINAL SOURCE: Synthetically Prepared

(vii) IMMEDIATE SOURCE: Synthetically Prepared

(viii) POSITION IN GENOME: None

(ix) FEATURE: None

(x) PUBLICATION INFORMATION: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

```
MSTNHRHNTM NNGNSNNNNV NVNKGVNCNV KNVNSGGGNV KNGGSNKNSC      50
AASGNTNSSY AMSWVRNTNN KRNNWVASNS SDGNTNYVDS VKGRNTVSRD     100
NARNNNYNNM SSNRSNDTAM YYCARNDYYG GGGNGYWGNG TNATVSAAST     150
KGNSVNNNAN SSKSTSGGTA ANGCNVKDYN NNNVTVSWNS GANTSGVHTN     200
NAVNNSSGNY SNSSVVTVNS SSNGTNTYNC NVNHKNSNTK VDKKVNNKSC     250
DKTHTCNNCN ANNNNGGNSV NNNNNKNKDT NMNSRTNNVT CVVVDASHND     300
NNVKNNWYVD GVNVHNAKTK NRNNNYNSTY RVVSVNTVNH NDWNNGKNYK     350
CKVSNKANNA NNNKTNSKAK GNNRNNNVYT NNNSRDNNTK NNVSNTCNVK     400
GNYNSDNAVN WNSNGNNNNN YKTTNNVNDS DGSNNNYSKN TVDKSRWNNG     450
NVNSCSVMHN ANHNHYTNKS NSNSNGK                               477
```

WE CLAIM:

1. A chimeric murine-human T84.12 antibody the kappa gene and the gamma gene of said antibody each having a murine variable region and a human constant region.

2. A chimeric murine-human T84.12 antibody kappa gene having a murine variable region and a human constant region.

3. A chimeric murine-human T84.12 antibody gamma gene having a murine variable region and a human constant region.

4. Isolated DNA having the sequence depicted by SEQ ID NO. 7 or SEQ ID NO. 9.

5. Isolated DNA having the sequence depicted by SEQ ID NO. 5.

6. SP2/0 myeloma cells cotransformed with expression vectors including SEQ ID NO. 3 and SEQ ID NO. 5.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/05709

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :A61K 39/395; C07H 21/04; C12N 5/10, 5/12; C12P 21/08
US CL :435/70.21, 172.2, 172.3, 240.27; 530/387.3; 536/23.53
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/70.21, 172.2, 172.3, 240.27; 530/387.3; 536/23.53

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, DIALOG, BIOSIS, EMBASE, MEDLINE, WPI

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US, A 5,081,235, (Shively et al.) 14 January 1992, see entire document.	1-6
X	EP,A, 0,337,746, (Beatty et al.) 18 October 1989, see entire document.	1-6
X	Cancer Research, Volume 51, issued 01 January 1991, P. Hutzell et al., "Generation and Characterization of a Recombinant/Chimeric B72.3", pages 181-189, see entire document.	1-6

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search	Date of mailing of the international search report
26 JULY 1993	16 AUG 1993
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	Authorized officer PHILLIP GAMBEL
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